Primer Island Transposition Kit

Protocol

PE Applied Biosystems
A DIVISION OF PERKIN-ELMER

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Introduction

Transposons

Transposons (TNs) are mobile genetic elements, regions of nucleic acid capable of inserting themselves or copies of themselves throughout the genome. Naturally occurring transposons encode the proteins that facilitate their movement into and out of DNA.

The Primer Island Transposition Kit is based upon the yeast Ty1 transposable element.Ty1 is a retrotransposon of the yeast *Saccharomyces cerevisiae*. In structure and mechanism it resembles retroviral proviruses. The Ty1 system is the only transposon for which random, high efficiency, *in vitro* integration has been described (Devine and Boeke, 1994). This *in vitro* transposition reaction is the basis of the Primer Island Transposition Kit.

The *in vitro* transposition system used in the Primer Island Transposition Kit places unique primer binding sites, “primer islands,” randomly throughout a population of large DNA molecules. These primer sites may be used subsequently for polymerase chain reaction (PCR) amplification or DNA sequencing reactions. Transposon insertion is an alternative to subcloning or primer walking when sequencing a large region of DNA (Devine and Boeke, 1994; Baker-Brachmann *et al.*, 1995; Devine *et al.*, 1997; Nam *et al.,* 1997; Williams *et al.,* 1997).

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How it Works

The Primer Island Transposition Kit provides the necessary reagents and control system for generating artificial transposon insertions into DNA *in vitro*. The Primer Island Kit reagents are combined and incubated with a target DNA, which is subsequently purified and electroporated into *Escherichia coli.*

The reagents in this kit have been optimized for transposon insertion into plasmid DNA. Insertion frequencies will vary according to the type and size of the DNA target.

To select for bacteria harboring a plasmid with a transposon insertion, the electroporation reaction is plated on Luria-Bertani (LB) agar plates containing the appropriate antibiotics (*i.e.,* one for the target plasmid and a second for the transposon).

The artificial transposon, AT-2, is included with the kit. In addition to the unique primer binding sites, AT-2 contains a gene that encodes dihydrofolate reductase (DHFR). DHFR confers resistance to trimethoprim (Fling and Richards, 1983). Assuming that the target plasmid encodes for the gene conferring ampicillin resistance, plating on ampicillin and trimethoprim LB-agar plates should permit the growth of only those bacteria containing a plasmid with a transposon insertion. Each ampicillin-resistant and trimethoprim-resistant colony will typically contain a plasmid with one copy of integrated transposon.

Insertions may be mapped by colony PCR amplification, or miniprep DNA may be isolated for DNA sequencing or PCR analysis.

For a more detailed explanation of how this technology works, refer to Devine and Boeke (1994), Devine *et al*. (1997), and Kimmel *et al*. (1997).

Benefits of This System

The Primer Island Transposition Kit has several advantages:

- Employs a simple *in vitro* reaction
- Uses any plasmid or *E. coli* host strain
- Inserts transposon randomly into diverse DNA sequences
- Inserts priming sites for PCR amplification
- Inserts priming sites for bidirectional DNA template sequencing

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Artificial Transposon

The linear transposon, AT-2, is shown in Figure 1 on page 4. This artificial transposon has the following features:

Feature	How displayed in Figure 1 on page 4	Function
Four-base pair termini	Labeled “U3”	Sequence recognized by Ty1 transposase. Allows the TN to be inserted efficiently into the target DNA.
DHFR gene[a]	Start and stop codons, GTG and TAA, respectively, are boxed	Codes for trimethoprim resistance.
Primer binding sites, PI(+) and PI(–)	Sequences are shaded: PI(+) extends from nucleotide 809–828. PI(–) extends from nucleotide 42–59.	During a sequencing reaction the primers are extended out into the adjacent DNA template, allowing the target DNA to be sequenced bidirectionally.
Primer binding sites, SD110 and SD111	Sequences are underlined	Allows amplification of PCR products using a single TN-specific primer in conjunction with a vector-specific primer.
Polylinker sequence	Boxed	Useful for restriction mapping of the site of transposon insertion prior to sequencing.

a. The gene was originally derived from Tn7 (Fling and Richards, 1983).

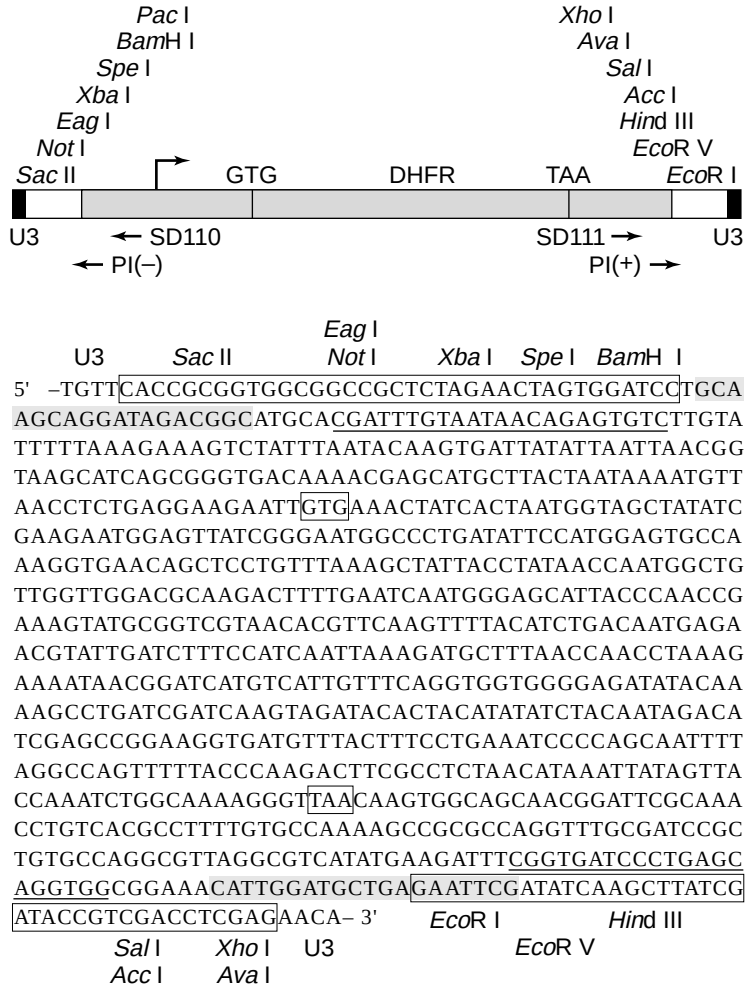

Figure 1 The artificial transposon AT-2. For description refer to “Artificial Transposon” on page 3. Figure adapted from Kimmel *et al*. (1997).

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Transposase Mechanism

The *in vitro* transposase reaction is catalyzed by a Ty1-encoded enzyme complex purified from *Saccharomyces cerevisiae* strain JB1143. This strain was modified from JB1141 (Devine and Boeke, 1994). The transposase (often referred to as an integrase in the literature) proceeds via a “cut-and-paste” transesterification mechanism to insert the transposon into the target DNA (Craigie, 1992). This process is shown in Figure 2. The DNA sequence shown in Figure 2 has been arbitrarily chosen.

During integration, the double-stranded target DNA is cleaved, resulting in a five-base pair staggered cut. The two blunt ends of the linear transposon DNA are simultaneously joined to the target DNA ends by single phosphodiester bonds. Because the reaction requires no additional energy source (*i.e.*, ATP), it is assumed that the energy produced by the strand cleavage is subsequently used to create the phosphodiester bond (Braiterman and Boeke,1994).

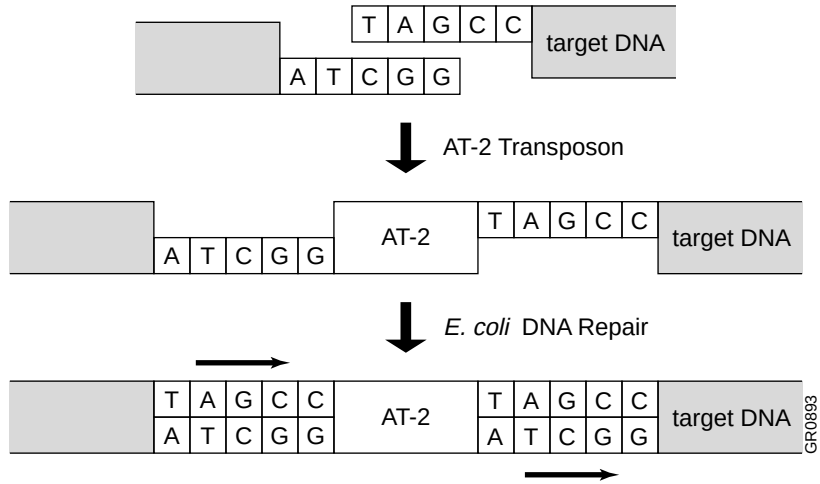

Figure 2 Transposase mechanism

The resulting intermediate DNA structure contains 5-bp single-stranded gaps that flank each end of the transposon. The gaps are filled in by an *E.coli* repair mechanism after transformation and are duplicated during the repair process. The short duplications generated are called “target-site duplications”.

The Primer Island Transposition Kit has been optimized to produce single transposon insertions in a plasmid target. The distribution of

transposition events will be randomly distributed over the target. The sequence of the DNA insertions can be manipulated using Factura™ and AutoAssembler™ software. The software removes vector, TN, and target-site duplication sequences and aligns overlapping DNA sequences.

Primers and Priming Sites

PI(+) and PI(–) Primers and Priming Sites

The primers, PI(+) and PI(–), bind to the priming sites that appear shaded on the AT-2 transposon figure (Figure 1 on page 4).

The sequences of the primers are:

PI(+): 5´-CAGGACATTGGATGCTGAGAATTCG-3´

PI(–): 5´-CAGGAGCCGTCTATCCTGCTTGC-3

Fluorogenic versions of these primers are available from PE Applied Biosystems as components of Primer Island Sequencing Kits. These kits are used in conjunction with the ABI PRISM™ Dye Primer Cycle Sequencing Core Kit. Refer to Appendix A on page 25 for part numbers.

These primers are similar to the SD118 and SD119 primers described by Boeke and Devine (1994). However, they have been modified by the addition of a mobility tag (CAGGA) to the 5´end of each oligonucleotide. In addition, primer-specific mobility files have been designed for these primers and are available on the Internet at the PE Applied Biosystems FTP site:

ftp://192.43.251.1/pub/public

SD110 and SD111 Nested Priming Sites

An additional set of primer binding sites, called SD110 and SD111, is located internal to the PI(+) and PI(–) sites. One of these sites can be used in conjunction with a vector-specific priming site to amplify PCR products. Sizing of these PCR products can be used to map the location of the TN insert in the vector. These PCR products can subsequently be sequenced using the PI(+) and PI(–) sequencing primers.

continued on next page

Control Target DNA

A plasmid control is included with the Primer Island Transposition Kit. This control is used to establish the frequency of transposon insertion, a measure of reagent performance. This plasmid consists of an insert of 527 base pairs from lambda DNA and confers ampicillin resistance. For the sequence of the plasmid, refer to the PE Applied Biosystems FTP site on the Internet. The address is referenced in “Primers and Priming Sites” on page 6.

Sequencing Control

A Sequencing Control has been included in the kit. This plasmid has binding sites for the PI(+) and PI(–) primers. For the sequence of the plasmid, refer to the PE Applied Biosystems FTP site. The address is referenced in “Primers and Priming Sites” on page 6.

Materials and Equipment

Kit Contents

The Primer Island Transposition Kit contains sufficient reagents for 20 transposition reactions:

Table 1. Kit Reagents

Reagent	Volume	Description
Transposase	40 µL	One vial containing the transposase enzyme complex in 10 mM HEPES-KOH, pH 7.8, 15 mM KCl, 5 mM EDTA, 50% sucrose
5X Transposase Buffer	200 µL	One vial containing 50 mM Tris, pH 7.5, 50 mM $MgCl_2$, 5 mM DTT, 25% PEG 8000
AT-2 Transposon	20 µL	One vial containing 200 µg/mL of purified transposon stored in 10 mM Tris, 1 mM EDTA, pH 8.0
Control Target DNA	25 µL	One vial containing 1 mg/mL of control plasmid stored in 10 mM Tris, 1 mM EDTA, pH 8.0
DNA Sequencing Control	34 µL	One vial containing 200 µg/mL purified, double-stranded, sequencing template. The template is stored in 10 mM Tris, 1 mM EDTA, pH 8.0

Note Refer to Appendix A on page 25 for descriptions and part numbers of kits closely related to the Primer Island Transposition Kit.

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Materials Recommended But Not Supplied

The items listed in Table 2 and Table 3 are recommended in addition to the reagents supplied with the Primer Island Transposition Kit.

Table 2. User-supplied equipment

Equipment Items	Source
ABI™ 373 DNA Sequencer, ABI PRISM 377 DNA Sequencer, or ABI PRISM 310 Genetic Analyzer	See your local PE Applied Biosystems representative for more information concerning these products.
Factura software	
AutoAssembler software	
1.5-mL microcentrifuge tubes	Major laboratory suppliers (MLS)
15-mL polypropylene screw-cap tubes	MLS
Adjustable volume pipettors, 0–20 µL, 20–200 µL, 100–1000 µL	MLS
Bunsen burner	MLS
Cell-Porator *E. coli* Electroporation System	Life Technologies, Inc. (P/N 11613-015)
Disposable gloves	MLS
Environmental shaker, 37 °C	MLS
Fine-bore gel loading pipette tips, filter plugged, 200 µL capacity	MLS
Incubator, 37 °C	MLS
Microcentrifuge	MLS
Microelecroporation chamber	Life Technologies, Inc. (P/N 11608-015)
Plate streaker	MLS
Pipette tips, with filter plugs	MLS
Rotating platform for uniform streaking of agar plates	MLS
Two heat blocks, 30 °C and 65 °C	MLS
Thermometers, 0–100 °C	MLS
Vacuum centrifuge	DuPont Speedvac or MLS

Table 3. User-supplied reagents

Reagent Items	Source
Ammonium acetate, molecular biology grade	Major laboratory suppliers (MLS)
Carbenicillin-LB agar plate[a]	Teknova, Inc.[b] (P/N 0133-C75) or MLS
Deionized water, nuclease and protease-free	MLS
EDTA, molecular biology grade	MLS
Electromax-competent, frozen *E. coli* cells, strain DH10B	Life Technologies, Inc. (P/N 18290-015)
Ethanol	MLS
Isopropanol	Fluka (P/N 59304)
Proteinase K	PE Applied Biosystems (P/N 400457)
S.O.C. Medium	Life Technologies, Inc. (P/N 15544-018)
SDS, molecular biology grade	MLS
Trimethoprim/Carbenicillin-LB agar plates[a]	Teknova, Inc. (P/N 0133-C75M75) or MLS

a. We prefer carbenicillin to ampicillin because of its stability properties. The ampR gene confers resistance to both antibiotics. If ampicillin is used, prepare fresh plates with 50 µg/mL ampicillin.

b. Teknova, Inc. (Half Moon Bay CA, 415-728-2557)

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Storage and Stability

Upon receipt, store the Primer Island Transposition Kit at –15 to –25 °C in a constant-temperature freezer. If stored under the recommended conditions, the product will maintain performance through the control date printed on the label.

Technical Support

In the United States or Canada, call (800) 831-6844 for technical support. Outside the United States and Canada, call you local sales office. Sales office numbers are given on the back cover of this protocol.

Safety

Please adhere to the following safety warnings.

5X Transposase Buffer

! WARNING ! CHEMICAL HAZARD. May cause skin, eye, and respiratory tract irritation. Wear gloves, a lab coat, and protective eyewear when handling. If contacted, wash affected area with large amounts of water for 15 minutes.

Other Chemicals

! WARNING ! CHEMICAL HAZARD. Certain chemicals used with this kit may be hazardous and require special handling. Do not store, handle, or work with any chemicals or hazardous materials unless you have received appropriate safety training and have read and understood all related Material Safety Data Sheets. Comply with all federal, state, and local laws related to chemical storage, handling and disposal.

Microorganisms

! WARNING ! Always follow CDC-NIH principles of biosafety for activities involving biological agents. These principles can be found in the U.S. Department of Health and Human Services Publication No. (CDC) 93-8395, *Biosafety in Microbiological and Biomedical Laboratories*.

Transposition, Electroporation, and Selective Plating

Transposition Reaction

During the transposition reaction, transposons are randomly integrated into the target DNA. The insertion is facilitated by a protein complex containing transposase. This reaction is described in detail by Devine and Boeke (1994) and by Kimmel *et al.* (1997).

IMPORTANT Target DNA purity is critical. Plasmid DNA should be purified by a standard method such as CsCl-gradient centrifugation (Sambrook *et al.*, 1989) or by column purification kits such as those sold by Qiagen or PE Applied Biosystems (P/N 401015). Miniprep DNA does not perform well in this reaction.

To complete the transposition reaction:

Step	Action
1	Thaw frozen reagents. When thawed, mix thoroughly by flicking the tube and inverting it several times.
2	Set up the following reaction in a 1.5-mL microcentrifuge tube. Reagents are listed in preferred order of addition: **Reagent** — **Volume (µL)** Deionized water — 12 5X Transposase Buffer — 4 AT-2 Transposon, 0.2 µg — 1 Control Target DNA, 1 µg or desired target DNA at 1 µg/µL — 1 Transposase — 2 Total volume — 20 **Note** The target DNA preparation can be resuspended in 1X TE or sterile, deionized water. Adjust the volume of deionized water in the reaction according to the volume of target DNA used. The total volume of deionized water and target DNA in the reaction is 13 µL.
3	Mix reagents gently. Do not vortex.
4	Incubate at 30 °C for one hour.
5	Prepare Stop Buffer immediately prior to use. Refer to Appendix B on page 26. **Note** Using anything other than freshly-prepared Stop Buffer may result in low electroporation efficiencies.

To complete the transposition reaction: *(continued)*

Step	Action
6	Stop the reaction by adding 5 µL of freshly-made Stop Buffer and incubating at 65 °C for 30 minutes.
7	Proceed to “Transposition Reaction Cleanup” on page 14.

continued on next page

Transposition Reaction Cleanup

Follow the steps below to precipitate, wash and resuspend the DNA.

To clean up the transposition reaction:

Step	Action
1	After 30 minutes at 65 °C, briefly centrifuge the samples to collect condensate at the tube bottom. Add the following: ♦ 25 µL deionized water ♦ 25 µL 7.5 M ammonium acetate ♦ 75 µL isopropanol **Note** Ethanol precipitation can be performed instead of isopropanol precipitation. Refer to Sambrook *et al.* (1989) for DNA precipitation methods.
2	Centrifuge immediately in a microfuge at 14,000 rpm for 30 minutes at 2–6 °C. **Note** Quantitative DNA recovery is essential for an adequate yield of trimethoprim-resistant (tmp^R) colonies. An excellent way to achieve this is to pellet the DNA to the bottom of the tube by using a rotor which secures the sample at a right angle with respect to the spindle axis. Alternatively, if a fixed angle rotor is used, be sure to resuspend the DNA by washing the side wall of the microcentrifuge tube extensively in step 6.
3	Carefully remove the supernatant with a 200 µL fine-bore pipet tip. Leave 5–10 µL of supernatant at the bottom of the tube. **Note** Do not allow the pipet tip to disturb the DNA pellet.
4	Rinse the pellet with 500 µL of ice-cold 70% ethanol and repeat step 2 and step 3.
5	Place the tube on the bench to air dry, or dry in a vacuum centrifuge for several minutes.
6	Resuspend the DNA pellet in 20 µL of sterile, deionized water. The final DNA concentration should be approximately 50 ng/µL. **IMPORTANT** Do not resuspend the DNA in 1X TE (10 mM Tris, 1 mM EDTA) or any conductive buffer.
7	Proceed to “Preparing for Electroporation” on page 15.

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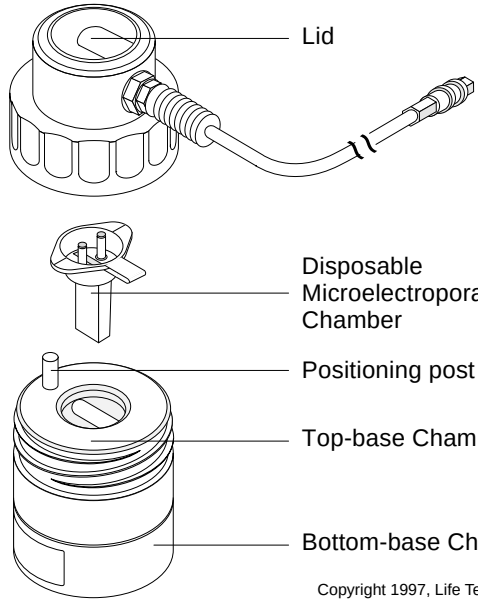
Preparing for Electroporation

After completing the transposition reaction and post-reaction DNA clean-up, prepare for the electroporation of the transposition reaction into electrocompetent DH10B *E. coli* cells.

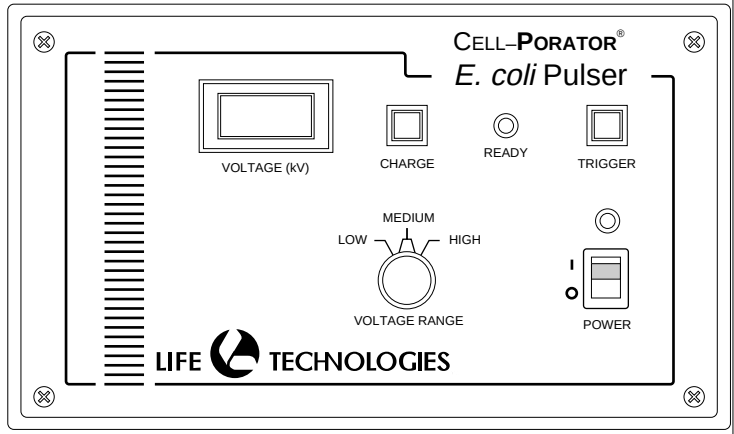
The procedures described below and in “Electroporation” on page 16 apply to use of an electroporator from Life Technologies, Inc. Similar devices from other manufacturers may also be used.

IMPORTANT All electroporation components must be chilled to 2–6 °C prior to contact with *E. coli* cells.

To prepare for electroporation:

Step	Action
1	Store the two-section base and the disposable microelectroporation chambers at 2–6 °C. Place on ice immediately before use.  Copyright 1997, Life Technologies, Inc. All rights reserved.
2	Prechill one microcentrifuge tube for each sample.
3	Separate the base into two components. Fill the top-base chamber with water and ice, then attach the bottom-base chamber. Place at 2–6 °C until ready to use. Allow the base to chill for at least 30 minutes before electroporation.

To prepare for electroporation: *(continued)*

Step	Action
4	Turn on the electroporator to allow time for warm up. Allow a 30-minute warmup before charging the capacitor. Set the voltage range to Medium.  Copyright 1997, Life Technologies, Inc. All rights reserved.
5	Place 1 mL of S.O.C. Medium into a 15-mL polypropylene tube. Leave on the bench top at room temperature. Prepare one tube for each sample.
6	Thaw the electrocompetent DH10B*E. coli* cells on ice, 15–25 minutes before use. **Note** Competent DH10B *E. coli* cells should be stored at –70 °C. A decline in electroporation efficiency is observed if cells remain thawed for an extended time period.
7	Proceed to “Electroporation.”

Electroporation

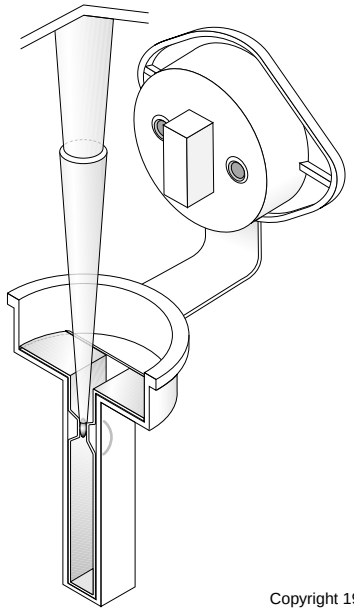
The procedure below describes the steps for electroporation.

IMPORTANT Poor transposition frequencies may result from low electroporation efficiencies.

To perform electroporation:

Step	Action
1	For each sample place 1 µL of chilled DNA and 16 µL of competent DH10B *E. coli* cells into a prechilled-microcentrifuge tube. Store the remainder of the transposition reaction at –15 to –25 °C.

To perform electroporation: *(continued)*

Step	Action
2	To monitor the efficiency of the electroporation, use the pUC 19 control plasmid provided with the electrocompetent DH10B *E. coli* cells. Dilute the pUC 19 control with sterile, deionized water to a concentration of 2.5 ng/µL. Place 1 µL of plasmid DNA and 16 µL of electrocompetent DH10B *E. coli* cells into a prechilled-microcentrifuge tube.
3	Check that the electroporator is charged. The voltage display should read approximately 2.45 kV.
4	Pipette 16 µL of the DNA + *E.coli* mixture into the gap between the two electrodes of the prechilled-microelectroporation chamber. Make sure the droplet does not contain any bubbles. Gently close the top. **Note** The droplet must remain in the electrode gap. Do not squeeze the body of the microelectroporation chamber, as this may alter the interelectrode distance. Handle the microelectroporation chamber at the rounded head.  Copyright 1997, Life Technologies, Inc. All rights reserved.
5	Place the disposable microelectroporation chamber into the chilled base.

To perform electroporation: *(continued)*

Step	Action
6	Place the lid on the base and tighten by clockwise rotation.
7	Press the Trigger (red button) of the electroporator.
8	Immediately pour 1 mL of S.O.C. Medium from the 15-mL polypropylene tube into the microelectroporation chamber, thus washing the droplet of DNA and *E. coli* from the electrode gap.
9	Pour the contents of the microelectroporation chamber back into the 15-mL polypropylene tube. Leave on the bench top at room temperature.
10	Repeat steps 3–8 until all of the samples and the pUC 19 controls have been electroporated and resuspended in S.O.C. Medium.
11	Shake the tubes at 225 rpm for 60 minutes at 37 °C.
12	Proceed to “Selective Plating.”

Selective Plating

Follow the procedure below for making serial dilutions of the electroporated DH10B cells and plating them on selective media. Two types of selective media will be used:

- Carbenicillin (75 µg/mL) in LB agar
- Carbenicillin and Trimethoprim (each at 75 µg/mL) in LB agar

Note This protocol provides guidelines for DH10B plating dilutions. The appropriate dilution will depend on your DNA sample. The goal is to select plating dilutions that will yield 100–300 colony-forming units (CFU) per plate.

To make dilutions and plate cells:

Step	Action
1	Prepare plating dilutions by setting up six 1.5-mL microcentrifuge tubes for each electroporated sample, except pUC 19 (see step 6). Label the dilution series as follows: ♦ 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}
2	Pipette 900 µL of S.O.C. Medium into each tube.
3	Pipette 100 µL of electroporated *E. coli* bacteria into the tube labeled “10^{-1}.” Gently invert the closed tube several times. Remove 100 µL from this tube and pipette the volume into the tube labeled “10^{-2}.”
4	Repeat mixing-and-pipetting routine until all tubes for a dilution series contain electroporated bacteria.

To make dilutions and plate cells: *(continued)*

Step	Action
5	Repeat this serial dilution procedure until each of the electroporated samples is serially diluted.
6	Prepare plating dilutions for the pUC 19 electroporation control by setting up five 1.5-mL microcentrifuge tubes. Label this dilution series as follows: ♦ 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}
7	Repeat steps 2–4 for the pUC 19 controls.
8	For each of the following dilutions, plate 100 µL of the transposition/DH10B electroporation mixture onto carbenicillin LB-agar plates: ♦ 10^{-5} ♦ 10^{-6} **Note** The agar plates should not be wet or have condensation on the lids. Prewarm the plates at 37 °C for 30 minutes prior to use. Plate in triplicate to ensure accuracy when assessing transposition frequency and electroporation efficiency.
9	For each of the following dilutions, plate 100 µL of the transposition/DH10B electroporation mixture onto carbenicillin/trimethoprim LB-agar plates: ♦ Undiluted ♦ 10^{-1}
10	For each of the following dilutions, plate 100 µL of pUC 19/DH10B electroporation mixture onto carbenicillin LB-agar plates: ♦ 10^{-4} ♦ 10^{-5}
11	Plate 100 µL of undiluted pUC 19/DH10B electroporation mixture onto carbenicillin/trimethoprim LB-agar plates.
12	Invert the plates and place them at 37 °C overnight. **Note** Discard all serial dilutions upon completion of step 10.
13	Proceed to “Data Analysis” on page 20.

Data Analysis

Transposition Data Analysis

Count bacterial colonies and determine the efficiencies and frequencies of transposition.

Step	Action
1	The following morning, count the number of colony-forming units (CFU) per LB-agar plate. **Note** Colonies plated on carbenicillin LB-agar plates grow faster than colonies plated on carbenicillin/trimethoprim LB-agar plates. Incubating plates for excessive time intervals will cause colonies to fuse, making them difficult to tabulate accurately. Colonies displaying a broad range of diameters may grow on carbenicillin/trimethoprim LB-agar plates. For an accurate CFU determination, count all colonies regardless of diameter.
2	Calculate the following as described on page 21: ♦ efficiency of transformation to carbenicillin resistance ♦ efficiency of transformation to carbenicillin/trimethoprim resistance ♦ frequency of transposition
3	Pick several colonies from the carbenicillin/trimethoprim plates and isolate the plasmid DNA using a standard small-scale isolation (miniprep) procedure.
4	Amplify the purified transformant DNA using PCR for mapping and/or sequencing, or immediately perform Cycle Sequencing as described in the *ABI PRISM Dye Primer Cycle Sequencing Core Kit Protocol* (P/N 402114).

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Transformation Efficiency

Transformation efficiency (T_F), or electroporation efficiency, is defined as the number of CFU of transformed bacteria produced per microgram of DNA subjected to electroporation.

Calculate the transformation efficiency for each electroporation using the formula:

$$T_F = \frac{\text{CFU}}{\text{ng DNA}} \times \frac{10^3 \text{ ng}}{\mu\text{g}} \times \begin{matrix}\text{Dilution}\\ \text{Factor}\end{matrix} \times \frac{1}{\text{Fraction of Dilution Plated}} = \frac{\text{CFU}}{\mu\text{g DNA}}$$

For example, DH10B is electroporated with 2.5 ng of pUC 19, and 1 mL of a 10^{-5} dilution of electroporated bacteria is prepared. A 100 µL plating of the 10^{-5} dilution yields 25 CFU. The transformation efficiency is calculated as follows:

$$T_F = \frac{25 \text{ CFU}}{2.5 \text{ ng}} \times \frac{10^3 \text{ ng}}{\mu\text{g}} \times 10^5 \times \frac{1}{(100\ \mu\text{L}/1\ \text{mL})} = 1 \times 10^{10} \frac{\text{CFU}}{\mu\text{g DNA}}$$

Frequency of Transposition

The Frequency of Transposition, F(Tp), is defined as the frequency of transposon insertion into the Control Target DNA and is expressed as the ratio of two transformation efficiencies:

- the efficiency of transforming DH10B to carbenicillin/trimethoprim resistance (with the target DNA containing at least one copy of the transposon)
- the efficiency of transforming DH10B to carbenicillin resistance

This ratio is expressed mathematically as follows:

$$F(Tp) = \frac{\text{Efficiency of carb}^R\text{, tmp}^R\text{ transformation}}{\text{Efficiency of carb}^R\text{ transformation}} = \frac{T_F\,(\text{carb}^R, \text{tmp}^R)}{T_F\,(\text{carb}^R)}$$

continued on next page

Five-Base Pair Repeats

As discussed in “Transposase Mechanism” on page 5, the insertion of the AT-2 transposon into the target DNA results in the generation of a five-base pair target-site duplication. This sequence flanks the transposon.

If the DNA is being sequenced by the ABI 373 or ABI PRISM 377 DNA Sequencer, then the raw data can be imported into Factura software to remove vector sequences from sequencing files. Factura software can also be used to recognize and remove the TN sequence from the sequencing file.

AutoAssembler software from PE Applied Biosystems can be used to align overlapping DNA sequences and remove 5-bp target-site duplications from the template sequence. These duplications provide a useful point to generate a “mini-contig” from each transposition.

Target DNA Deletions

A deletion of a segment of the target DNA occurs infrequently during transposon insertion. The reasons for deletion of target DNA are not known, but appear to be target DNA sequence dependent.

There are two indications of a target DNA deletion:

- The typical 5-bp target-site duplication is not observed. (Refer to Figure 2 on page 5 for transposase mechanism).
- The sequences from both ends of the transposon do not align as expected during the final sequence assembly. The sequences may be separated by ten base pairs to several kilobases, depending upon the size of the target DNA deletion.

When a target DNA deletion occurs, the sequence data obtained from each end of the transposon is valid. There have been no reports of internal deletions or scrambling of sequences by the transposon. The two sequences may be considered separately and assembled accordingly.

continued on next page

Performance Specifications

The performance specification is based upon the frequency of transposition, F(Tp). Using control reagents and protocol, the F(Tp) value should equal or exceed 1×10^{-5}.

That is, for every 10^9 carbenicillin-resistant transformants recovered, at least 10^4 transposition events should be recovered.

Note Inefficient electroporation will adversely impact the yield of trimethoprim-resistant colonies.

Twenty nanograms of Control Target DNA recovered from the transposition reaction should yield 100–300 carbenicillin-resistant, trimethoprim-resistant bacterial colonies.

Troubleshooting

Observation	Possible Cause	Recommended Action
Poor recovery of tmp^R bacterial colonies and the transformation efficiency of the pUC 19 control is $>1 \times 10^{10}$ CFU/µg or The transformation efficiency of bacteria plated on carbenicillin ($carb^R$) is at least a factor of 10^6 greater than the transformation efficiency of bacteria plated on carbenicillin/trimethoprim ($carb^R/tmp^R$)	DNA impurities	Establish the transposition frequency empirically, using your reagents with the Control Target DNA. Compare with the transposition frequency obtained using your template DNA.
		Purify plasmid DNA using column chromatography or CsCl centrifugation. Mini-prepped DNA is not recommended.
		Electrophorese cut and uncut target DNA (overloaded) on an agarose gel to screen for contaminants.
	DNA contaminated with protein	Extract the transposition reaction with phenol/chloroform. Precipitate the DNA using isopropanol or ethanol followed by a 70% ethanol wash.
No tmp^R colonies recovered and the transformation efficiency of the pUC 19 control is $<8 \times 10^9$ CFU/µg	Inadequate recovery of DNA pellet	Following precipitation of the transposition reaction, carefully resuspend all of the DNA by washing the walls of the tube.
	Incorrect electroporator settings or poor technique	Check electroporator settings. Repeat pUC 19 electroporation after reviewing the troubleshooting guide in the electroporator user's manual.
Bacterial "background" growth on LB-agar plates is too high	Bad agar plate or aggressive bacterial growth	Switch to M9-agar plates to reduce growth rates.

Appendix A. Primer Island Kits and Related Sequencing Kits

Name	P/N	Description
Primer Island Transposition Kit	403015	Contains reagents necessary to perform 20 transposition reactions. Includes the protocol.
Primer Island Transposition Kit	402984	Contains reagents necessary to perform 20 transposition reactions. Does not include the protocol.
Primer Island Transposition Kit Protocol	402920	
Primer Island(+) Dye Primers	402983	Contains the PI(+) primers only, sufficient for 50 sequencing reactions. Primers are labeled with 5-FAM, JOE, TAMRA, and ROX.
Primer Island(–) Dye Primers	402982	Contains the PI(–) primers only, sufficient for 50 sequencing reactions. Primers are labeled with 5-FAM, JOE, TAMRA, and ROX.
Primer Island(+) Sequencing Kit	403014	Contains reagents necessary to perform 100 sequencing reactions. Contains the PI(+) primers and P/N 402070. Primers are labeled with 5-FAM, JOE, TAMRA, and ROX.
Primer Island(–) Sequencing Kit	403013	Contains reagents necessary to perform 100 sequencing reactions. Contains the PI(–) primers and P/N 402070. Primers are labeled with 5-FAM, JOE, TAMRA, and ROX.
Primer Island(+/–) Sequencing Kit	402980	Contains reagents necessary to perform 100 sequencing reactions. Contains the PI(+) and PI(–) primers and P/N 402070.
ABI PRISM Dye Primer Cycle Sequencing Core Kit Protocol	402114	
ABI PRISM Dye Primer Cycle Sequencing Core Kit with AmpliTaq® DNA polymerase, FS	402125	Contains reagents to perform 100 sequencing reactions. Does not contain primers. Includes the protocol.
ABI PRISM Dye Primer Cycle Sequencing Core Kit with AmpliTaq DNA polymerase, FS	402070	Same reagents as P/N 402125 above. Does not include the protocol.

Appendix B. Preparing Stop Buffer

Procedure The table below describes how to prepare the Stop Buffer used in the "Transposition Reaction" on page 12.

Step	Action
1	Combine the following: **Reagent** / **Volume (µL)** Deionized water — 27.5 0.5 M EDTA — 50.0 10% SDS — 10.0 40 mg/mL Proteinase K — 12.5 Total volume — 100.0 **Note** The Proteinase K should be suspended in 10 mM $CaCl_2$, aliquoted and frozen at –70 °C. Under these storage conditions, the stock is usable for months. Do not reuse thawed aliquots.
2	If a precipitate appears in the Stop Buffer, warm the bottom of the microcentrifuge tube by gently rubbing it with your fingers.
3	Gently mix the tube. Do not vortex.

Appendix C. References

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